

PATENT
UTSC:669US

APPLICATION FOR UNITED STATES LETTERS PATENT
for
NOVEL ANTISEPTIC DERIVATIVES WITH BROAD SPECTRUM
ANTIMICROBIAL ACTIVITY FOR THE IMPREGNATION OF SURFACES
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BACKGROUND OF THE INVENTION

The present invention claims priority to U.S. Provisional Application Serial No. 60/261,447 filed on January 12, 2001 and U.S. Provisional Application Serial No. 60/316,165 filed on August 30, 2001, which are incorporated by reference in their entirety.

1. Field of the Invention

The present invention relates generally to the fields of preventing infections. More particularly it provides novel broad spectrum antiseptic compositions that further have properties that allow them to be coated/impregnated into polymer surfaces or used as antiseptics in different applications. The invention provides methods for coating these antiseptic compositions onto medical devices such as catheters, tubes, stents and sutures, to prevent the growth of pathogens in such devices and hence, to prevent infection to patients via such devices. In addition, the invention provides novel antiseptics that could be used in disinfecting and sterilizing organic and inorganic surfaces, water and other fluids.

2. Description of Related Art

Most nosocomial infections are caused by the contamination of medical devices resulting in serious hospital-acquired infections. Nosocomial pneumonias are the second most common nosocomial infections, and are associated with the highest attributable mortality and morbidity. Recent data have shown that at least 300,000 episodes of nosocomial pneumonia occur annually in the United States (Official Statement, American Thoracic Society). The attributable mortality of this infection is 33%-50%, hence, around 100,000 patients die annually because of nosocomial pneumonia (CDC, 1993; Leu *et al.*, 1989). The risk of nosocomial pneumonia increases 6- to 20-fold from the use of mechanical ventilation (Official Statement, American Thoracic Society).

The endotracheal tube is considered a common vehicle for colonization/contamination leading to nosocomial pneumonia. The endotracheal tube connects the oropharyngeal environment with the sterile bronchoalveolar space, significantly increasing the risk of nosocomial pneumonia. Endotracheal tubes are

typically constructed of polyvinylchloride, which is known to be very difficult to impregnate with antiseptic or antimicrobial agents. Thus, there are no endotracheal tubes that are impregnated with antibiotics or antiseptics currently in use.

Another leading cause of serious nosocomial infections is bloodstream infections.

5 The primary contributors to nosocomial bloodstream infections are vascular catheters. It is estimated that around 400,000 vascular catheter-related bloodstream infections (CRBSI) occur annually in the United States (Raad, 1998). The attributable mortality of these infections in the intensive care unit (ICU) was estimated in JAMA in 1994 to be 25% (Reiselman *et al.*, 1994). Hence, these infections are a major cause of morbidity and
10 mortality in hospitalized patients. These catheters are mostly polyurethane short-term catheters used in the ICU and long-term silicone catheters used in cancer/AIDS patients.

The most frequent causes of nosocomial infections are urinary tract infections (UTI), contributing to 34% of all nosocomial infections (Klempner *et al.*, 1998).

Nosocomial UTI are usually associated with contamination of urinary catheters. In
15 addition, nosocomial surgical wound infections are common complications of surgical procedures, particularly in cancer and immunocompromised patients with devitalized tissue and decreased immunity. Surgical wound infections contribute to 17% of all nosocomial infections (Platt and Bucknall, 1988). Many surgical wound infections are associated with the contamination of sutures.

20 Antibiotics are strictly antibacterial agents that are usually used in treatment of systemic or bloodstream infections and are given through oral, intravenous, subcutaneous, or intramuscular routes to achieve systemic bloodstream levels. Examples include penicillin, cephalosporins, vancomycin, minocycline, and rifampin.

Antiseptics on the other hand, are antimicrobial agents often with broad spectrum
25 antimicrobial activity against bacteria, fungi or viruses. These agents are used on the skin and external mucosal surfaces usually because of limitations related to absorption, penetration or systemic toxicity. These agents are not used in the treatment of bloodstream infections. Examples include chlorhexidine and povidone iodine.

30 Antibiotics and antiseptics have been used to impregnate vascular catheters. The concern with the use of antibiotics has been that resistance might develop to antibiotics, preventing their use therapeutically and systemically in hospitalized patients.

Furthermore, the durability of the existing antiseptics has been limited. For example, the use of chlorhexidine/silver sulfadiazine on polyurethane surfaces has had limited effectiveness. Moreover, chlorhexidine/silver sulfadiazine impregnating the surface of vascular catheters resulted in limited activity against gram-negative bacilli, such as 5 *Pseudomonas*.

What is needed is an effective antiseptic having broad spectrum activity against 10 resistant staphylococci, vancomycin-resistant enterococci, resistant *Pseudomonas aeruginosa* and *Candida* species, to be used in conjunction with indwelling devices that will inhibit or prevent the nosocomial infections typically associated with the use of these 15 indwelling devices. It would be further desirable to develop devices impregnated with the antiseptic to enhance the resistance to infection. For example, the creation of antiseptic-impregnated catheters would prevent organisms from adhering or migrating on catheter surfaces.

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SUMMARY OF THE INVENTION

The present invention overcomes these and other drawbacks inherent in the art by providing novel antiseptic derivatives with broad-spectrum activity against various microbes including resistant bacteria and fungi. Methods of preparing these antiseptic compounds and methods for utilizing them are provided.

20 Therefore, the invention provides an antiseptic composition comprising a basic reagent and a dye. The basic reagent may be bonded to the dye. In one aspect, the basic reagent and the dye are bonded ionically to form the antiseptic compound. In another aspect, the basic reagent and the dye are bonded covalently to form the antiseptic compound. The basic reagent and the dye can be combined in any amount to obtain the 25 antiseptic composition of the invention, however, in a particular embodiment, an equimolar amount of the basic reagent is added to the dye solution. The inventors also contemplate that the antiseptic composition of the invention can be made by combining other amounts of the dye and basic reagent for example, one may combine, in molar ratios, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 30 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1.85, 1:90, 1:95, to 1:99 of either dye : basic reagent or basic reagent : dye. This includes all the intermediate ranges as well, for

example it includes molar ratios such as, 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1 and the like for other values listed. It also includes the ranges in between these values such as 1.11:1, 1.12:1 and so on. The skilled artisan will therefore recognize that the dye and basic reagent can be combined in different molar ratio amounts to obtain 5 the antiseptic composition disclosed and that the invention is therefore not limited to any particular molar ratio of dye : basic reagent or basic reagent : dye.

In certain embodiments, the dye can be a triarylmethane dye, a monoazo dye, a diazo dye, an indigoid dye, a xanthene or a fluorescein dye, an anthraquinone dye, or a quinoline dye. In other specific embodiments, the dye is gentian violet, or crystal violet, 10 ethyl violet, brilliant green, an FD&C dye, or a D&C dye. In one example, the FD&C dye is Blue No. 1 or Green No. 3. In another example, the triarylmethane dye is gentian violet. In yet another example, the monoazo dye is FD&C Yellow No. 5 or FD&C Yellow No. 6. In still another example, the diazo dye is D&C Red No. 17. The indigoid dye, may preferably be FD&C Blue No. 2. An example of a xanthene dye is FD&C Red 15 No. 3, of an anthraquinone dye is D&C Green No. 6 and an example of a quinoline dye is D&C Yellow No. 1. In addition, Table 1 provides a list of different dyes that may be used in this invention. One of skill in the art will recognize that these examples are non-limiting and that the antiseptic compounds and compositions of the present invention can be made using almost any dye.

20 A wide variety of basic reagents can be used to form the antiseptic composition. The basic reagents include any nucleophilic species which includes all electron donor species. Some of the basic reagents that can be used include a guanidium compound, a biguanide, a bipyridine, a phenoxide antiseptic, an alkyl oxide, an aryl oxide, a thiol, an aliphatic amine, or an aromatic amine and halides such as F⁻, Br⁻ and I⁻. Some examples 25 of guanidium compounds that may be used include chlorhexidine, alexidine, and hexamidine. One example of a bipyridine compound that can be used to synthesize the antiseptics of the invention is octenidine. Examples of phenoxide antiseptics used include colofoctol, chloroxylenol, and triclosan.

30 The invention also provides an antiseptic compound comprising a basic reagent bound to a dye. In some particular embodiments, the basic reagent and the dye are bound ionically. In other particular embodiments, the basic reagent and the dye are bound

covalently. Some examples of the invention, the antiseptic compound comprises compositions such as gendine, genlenol, genlosan, or genfocitol.

In some embodiments, the antiseptic compound provided herein is further capable of impregnating and/or coating a surface.

5 In some aspects, the surface is composed of a polymer. Examples of such polymeric surfaces include polyvinyl chloride, polyurethane, polyethylene, silastic elastomers, polytetrafluoroethylene, dacron, collodion, carboethane or nylon. Alternatively, the surface may be composed of silicone or may be a silk suture. For example, these novel antiseptic derivatives have the potential for serving as impregnators 10 of medical device surfaces, such as endotracheal tubes made of polyvinyl chloride, vascular catheters made of either polyurethane or silicone, and silk sutures used for suturing surgical wounds.

In other embodiments, the antiseptic compound can coat and/or impregnate an 15 organic surface. Examples of organic surfaces include the skin, a mucosal surface, a wound. Examples of wounds are surgical wounds, trauma wounds, burn wounds and the like.

In yet other embodiments, the antiseptic compound can coat and/or impregnate an 20 inorganic surface. Examples of such inorganic surfaces include floors, table-tops, counter-tops, surfaces of a hospital equipment, wheelchair surfaces, *etc.* Virtually any surface comprising a material that is capable of being coated by, impregnated with, absorbing or otherwise retaining the antiseptic compounds of the invention may be disinfected and/or sterilized using the present antiseptic compounds and their compositions. Thus, the antiseptic compound of the invention can be used to disinfect, 25 sanitize and sterilize a wide variety of surfaces.

25 The invention also provides medical devices coated with a basic reagent and a dye. In one aspect the medical devices are coated with a basic reagent and a dye that are ionically bound. In another aspect the medical devices are coated with a basic reagent and a dye that are covalently bound. Examples of medical devices include endotracheal tubes, a vascular catheter, an urinary catheter, a nephrostomy tube, a biliary stent, a peritoneal 30 catheter, an epidural catheter, a central nervous system catheter, an orthopedic device, a prosthetic valve, and a medical implant. The vascular catheter may be a central venous

catheter, an arterial line, an pulmonary artery catheter, and a peripheral venous catheter. The central nervous system catheter may be an intraventricular shunt. Other medical devices that can benefit from the present invention include blood exchanging devices, vascular access ports, cardiovascular catheters, extracorporeal circuits, stents, implantable 5 prostheses, vascular grafts, pumps, heart valves, and cardiovascular sutures, to name a few. Regardless of detailed embodiments, applicability of the invention should not be considered limited with respect to the type of medical device, implant location or materials of construction of the device.

The invention also provides methods for coating a medical device with an 10 antiseptic compositions comprising: a) immersing a medical device in a solvent comprising a basic reagent and a dye; b) drying the device; and c) washing off excessive composition. In some embodiments, the solvent used to immerse the device can be methylene chloride, methanol, or a combination thereof.

The invention also provides methods for preventing nosocomial infections in a 15 subject comprising coating a medical device that the subject is required to use with a composition comprising an antiseptic compound comprising a basic reagent bound to a dye. The subject can be human or an animal model.

The type of nosocomial infection that can be prevented by the methods of this 20 invention include, but are not limited to, pneumonia, bacteremia, fungemia, candidemia, a urinary tract infection, a catheter-exit site infection, and a surgical wound infection.

The nosocomial infections that can be prevented may be caused by bacteria. In some embodiments the bacteria are drug resistant bacteria. Some non-limiting example of drug resistant bacteria include methicillin-resistant staphylococci, vancomycin-resistant enterococci, and resistant *Pseudomonas aeruginosa*.

25 The nosocomial infection may be caused by a fungus. In some cases the fungal agent is a drug resistant fungi. Examples of a drug resistant fungi include members of the *Candida species*. Other pathogenic organisms that can cause the nosocomial infections are cited elsewhere in this specification and coating devices and surfaces with the antiseptics of the present invention can prevent infections by these organisms as well.

30 The invention also provides methods for disinfecting and/or sterilizing a surface comprising applying the antiseptic composition of the invention to the surface. Examples

of surfaces that may be disinfected and/or sterilized include organic surfaces such as skin, mucosal surfaces, wound surfaces and the like. Other examples include inorganic surfaces such as floors, table-tops, counter-tops, hospital equipment, wheel chairs, gauze, cotton. The skilled artisan will realize that most any surfaces can be disinfected or 5 sterilized by the antiseptic compositions provided herein.

The invention also provides methods for disinfecting and/or sterilizing a fluid comprising adding a composition comprising a basic reagent and a dye into the fluid. The inventors contemplate that different types of fluids may be disinfected and some non-limiting examples include water, such as water in coolers and swimming pools, 10 metal working fluids, and petroleum.

The invention also provides several novel biocide preservative compounds comprising the antiseptic compositions described above. Also provided are methods for preserving substances by applying the compositions of the invention on the substance. A variety of substances may be preserved by the biocide preservatives of the invention and they include wood, paint, plastic and paper. 15

Thus, the antiseptic compositions of the present invention have broad uses including use in healthcare by providing sterile medical devices and surface sterilization and decontamination, as environmental decontaminants, fluid decontaminants and in the industrial world as biocide preservatives.

20 As used herein the specification and claim(s), the words "a" or "an" when used in conjunction with the word "comprising" may mean one or more.

As used herein the specification and claim(s), the words "ionic bonding" or "ionically bound" refers to the electrostatic interactions among ions which can be formed by the transfer of one or more electrons from one atom or group of atoms to another, to 25 create an ionic bond between the basic reagent and the dye comprising an antiseptic compound.

As used herein the specification and claim(s), the words "covalent bonding" or "covalently bound" refers to the chemical bond formed by the sharing of one or more pairs of electrons between the basic reagent and the dye comprising an antiseptic 30 compound.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various 5 changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

10 A. The Present Invention

Indwelling catheters and other similar implanted medical devices are used routinely in hospitals on a diverse group of patients. A common cause of failure of these medical devices is infection. Pathogens often attach to and proliferate in such devices and eventually invade the patient leading to nosocomial infections. Microorganisms 15 usually migrate along the surfaces of devices to invade sterile environments, such as the bronchoalveolar space leading to pneumonia, the bloodstream leading to bacteremia, or the urinary bladder leading to urinary tract infections.

The present invention provides a series of novel antiseptic compositions with broad-spectrum activity against various nosocomial microorganisms, including resistant 20 bacteria and fungi. For example, the antiseptic compositions are effective against resistant staphylococci, vancomycin-resistant enterococci, resistant *Pseudomonas aeruginosa* and *Candida* species. These novel antiseptics also have unique properties that enable penetration/impregnation of various polymers, such as polyvinyl chloride, 25 polyethylene, silastic elastomers, polytetrafluoroethylene, dacron, collodion, carboethane, nylon, polymers used in the formation of endotracheal tubes, silicone and polyurethane polymers used in the formation of vascular catheters and surgical silk sutures. Thus, they are suitable for coating a wide range of device surfaces.

The inventors demonstrate herein that these novel antiseptics maintain prolonged 30 antimicrobial activity on device surfaces, and thus may be used for the entire lifespan of these indwelling devices. This is an improvement over existing coated or impregnated devices where the antimicrobial activity of the device diminishes over time and eventually

disappears altogether. For example, several prior art patents and publications describe methods of coating which generated medical devices wherein the effectiveness of the coating diminishes over time. After insertion of the medical device, the antibiotics and/or antiseptics quickly leach from the surface of the device into the surrounding environment.

5 Over a relatively short period of time, the amount of antibiotics and/or antiseptics present on the surface decreases to a point where the protection against bacterial and fungal organisms is no longer effective. Thus, the present invention provides safe antiseptic treated devices wherein the antiseptic coating has a durability that may last through the life-span of the device. This significantly decreases patient mortality and morbidity
10 associated with the various nosocomial infections such as nosocomial pneumonias, nosocomial bacteremias, nosocomial urinary tract infections and nosocomial surgical wound infections. For example, creation of antiseptic-impregnated/coated catheters prevents organisms from adhering to or migrating on catheter surfaces. Thus, when a pathogenic organism approaches the catheter surface, it is killed by the antiseptics.

15 The invention also provides methods for the synthesis of novel broad-spectrum antiseptic derivatives. The general method for synthesis of the novel antiseptic derivatives involves the binding of a dye with one or more basic reagents. Different types of dyes and basic reagents can be used to prepare the antiseptic compounds of this invention.

20 The dyes that may be used to synthesize the antiseptic compounds of the invention include but are not limited to, gentian, or crystal violet, ethyl violet, brilliant green, *etc.*, and the FD&C dyes such as Blue No. 1 and Green No. 3. In addition, other dyes include the following FD&C and D&C colors: (1) Monoazo dyes such as, but not limited to, FD&C Yellow No. 5, FD&C Yellow No. 6, (2) Diazo dyes such as, but not limited to, D&C Red No. 17, (3) Indigoid dyes such as, but not limited to, FD&C Blue
25 No. 2, (4) Xanthene (Fluorescein) dyes such as, but not limited to, FD&C Red No. 3, (5) Anthraquinone dyes such as, but not limited to, D&C Green No. 6, (6) Quinoline dyes such as, but not limited to, D&C Yellow No. 1. An extensive list of dyes and stains that may be employed is also provided in Table 1.

30 The basic reagents can be alkyl and aryl oxides, thiols, sulfides, phosphorous, aliphatic and aromatic amines, guanidines and halides such as F⁻, Br⁻ and I⁻. Some examples

of the basic reagents that can be used include phenoxide antiseptics (such as clofoctol, chloroxylenol, triclosan) or guanidium compounds (such as chlorhexidine, alexidine, hexamidine) or bipyridines (such as octenidines).

5

Table 1. The Color Index (C.I.) Number and/or Chemical Abstracts Service Registry CAS) Number for Dyes and Stains that may be Employed to Stain Medical Devices:

No.	C.I. #	CAS #	No.	C.I. #	CAS #
1	15670	2092-55-9	51	65005	1328-24-1
2	26370	3071-73-6	52	62055	6408-78-2
3	20460	5850-35-1	53	62125	6424-85-7
4	62130	2666-17-3	54	63010	2861-02-1
5	61585	4474-24-2	55	13390	3861-73-2
6	26360	3351-05-1	56	26400	3529-01-9
7	62058	6397-02-0	57	15706	12392-64-2
8	42685	3244-88-0	58	61570	4403-90-1
9	61580	6408-57-7	59	62560	4430-16-4
10	15575	5850-86-2	60	26550	8003-88-1
11	22870	15792-50-4	61	18745	10127-27-2
12	18050	3734-67-6	62	14710	5858-39-9
13	14900	4787-93-3	63	17045	6360-07-2
14	18070	12167-45-2	64	15620	1658-56-6
15	22890	10169-02-5	65	18110	6844-74-2
16	23635	6459-94-5	66	26900	6406-56-0
17	18800	6408-31-7	67	18125	10130-48-0
18	18055	4321-69-1	68	42650	4129-84-4
19	18965	6359-98-4	69	18835	6359-85-9
20	18900	6359-91-7	70	18890	6359-90-6
21	25135	13390-47-1	71	18950	6372-96-9
22	22910	6375-5-9	72	14170	6408-90-8
23	18850	6359-88-2	73	13900	10343-58-5
24	46005:1	494-38-2	74	46025	135-49-9
25		8048-52-0	75	12840	61968-76-1
26	58000	72-48-0	76	63615	1324-21-6
27		3952-78-1	77	58005	130-22-3
28	61710	6408-63-5	78	14025	584-42-9
29	42750	30586-13-1	79	42080	3486-30-4
30		569-58-4	80	16185	915-67-3
31		52417-22-8	81	42780	
32		520-10-5	82		1668-00-4
33	48035	3056-93-7	83	41000	2465-27-2
34		4431-00-9	84	43810	13186-45-3
35	50090	25360-72-9	85	52005	531-53-3
36	52010	531-55-5	86	51004	33203-82-6
37	61111	12217-43-5	87	11075	94233-04-2
38	42500	569-61-9	88	42510	632-99-5
39	11460	42373-04-6	89	48055	4208-80-4
40	23500	992-59-6	90	26905	4196-99-0
41		298-95-3	91		2315-97-1
42	21010	5421-66-9	92	21000	10114-58-6

43		1871-22-3	93	16180	5858-33-3
44	28440	2519-30-4	94	42655	6104-58-1
45	42660	6104-59-2	95		81029-05-2
46	27290	5413-75-2	96	42040	633-03-4
47	24890	3051-11-4	97		102185-52-4
48		76-60-8	98		62625-32-5
49		115-40-2	99		62625-30-3
50		115-39-9	100		62625-28-9
101		14337-53-2	152		16574-43-9
102		76-59-5	153		34722-90-2
103		40070-59-5	154		617-19-6
104		3147-14-6	155	51050	1562-90-9
105	24410	2610-05-1	156		4430-20-0
106	43825	1667-99-8	157	14720	3567-69-9
107	16575	548-80-1	158	16570	4197-07-3
108	43820	3564-18-9	159	11270	532-82-1
109	24895	2870-32-8	160	18105	17681-50-4
110	18972	50662-99-2	161	22120	573-58-0
111		596-27-0	162		2411-89-4
112		2303-01-7	163		62625-31-4
113		1733-12-6	164		62625-29-0
114		10510-54-0	165		41830-80-2
115	15970	1934-20-9	166	42555	548-62-9
116		15391-59-0	167	45370:1	596-03-2
117		76-54-0	168		620-45-1
118		4727-50-8	169	45425:1	31395-16-1
119		54-88-6	170		73688-85-4
120		6473-13-8	171	34140	4399-55-7
121	23655	6420-03-7	172	29160	3441-14-3
122	25380	2829-43-8	173	28160	2610-11-9
123	27905	5489-77-0	174	13920	10130-29-7
124	13950	10190-68-8	175	19556	6537-66-2
125	29025	3214-47-9	176	36900	6409-90-1
126	64500	2475-45-8	177	61505	2475-46-9
127	61500	2475-44-7	178	11080	2581-69-3
128	1005	730-40-5	179	26080	6253-10-7
129		31482-56-1	180	11110	2872-52-8
130	11115	3180-81-2	181	11130	2734-52-3
131	11855	2832-40-8	182	12790	6439-53-8
132	26090	6300-37-4	183		518-82-1
133	45400	548-24-3	184		56360-46-4
134	45380	548-26-5	185	45380:2	15086-94-9
135		15086-94-9	186	14645	1787-61-7
136	14640	3564-14-5	187	18760	3618-63-1
137	42090	3844-45-9	188	45430	568-63-8
138	45430:2	15905-32-5	189		1239-45-8
139	45386	6359-05-3	190		62758-12-7
140		76058-33-8	191	42600	2390-59-2
141	23860	314-13-6	192	37190	64071-86-9
142	11160	97-56-3	193	42053	2353-45-9
143	13015	2706-28-7	194	12010	6535-42-8
144	11285	6416-57-5	195	18820	6359-82-6
145	45350:1	2321-07-5	196	45350	518-47-8

146		596-09-8	197		3326-32-7
147		3326-34-9	198		51649-83-3
148	51030	1562-85-2	199	42085	4680-78-8
149		1634-82-8	200	75290	517-28-2
150		3737-95-9	201		90-33-5
151		165660-27-5	202	73000	482-89-3
203	73015	860-22-0	254		3599-32-4
204	12210	4569-88-4	255		146-68-9
205	11050	2869-83-2	256	42095	5141-20-8
206	44090	3087-16-9	257	42000:1	510-13-4
207	42000	2437-29-8	258		129-16-8
208	13065	587-98-4	259	52015	61-73-4
209	52041	2516-05-4	260	50206	4569-86-2
210	45385	23391-49-3	261	42590	7114-03-6
211	13025	547-58-0	262	13020	493-52-7
212		32469-43-5	263	11020	60-11-7
213	14855	3624-68-8	264	20110	3564-15-6
214	11335	6247-27-4	265	11875	6247-28-5
215	11880	6370-46-3	266	13250	3618-62-0
216	11300	6232-53-7	267	14030	2243-76-7
217	26520	3564-27-0	268	26560	6406-37-7
218	18735	1934-24-3	269		6408-91-9
219	14010	6054-99-5	270	14045	6470-98-0
220	44530	5715-76-4	271	20470	1064-48-8
221	11350	131-22-6	272	50040	553-24-2
222	16255	2611-82-7	273	42520	3248-91-7
223	52030	6586-05-6	274	51180	3625-57-8
224		7385-67-3	275	14890	5423-07-4
225		74-39-5	276		56431-61-9
226	60760	6409-77-4	277	61555	2646-15-3
227	26120	4477-79-6	278	26125	1320-06-5
228	16230	1936-15-8	279	15510	633-96-5
229	15705	2538-85-4	280	15711	5610-64-0
230	19010	10127-05-6	281	12070	6410-10-2
231	42045	129-17-9	282		143-74-8
232		34487-61-1	283	11000	60-09-3
233		101-75-7	284		16201-96-0
234	11800	1689-82-3	285		975-17-7
235	45410	18472-87-2	286		2768-90-3
236	16680	1058-92-0	287	27195	6226-79-5
237	27190	6226-78-4	288		67627-18-3
238	49000	30113-37-2	289	58205 (75410)	81-54-9
239		16593-81-0	290		115-41-3
240		85531-30-2	291	45010	2150-48-3
241	45005	92-32-0	292		117-92-0
242	58500	81-61-8	293	58050	81-64-1
243	47000	8003-22-3	294	47005	8004-92-0
244	20505	17095-24-8	295	61211	12236-82-7
245	61205	13324-20-4	296	17757	12225-82-1
246	17908	25489-36-5	297	61200	2580-78-1
247		635-78-9	298		123333-76-6
248	45170	81-88-9	299	45170:1	509-34-2
249	45160	989-38-8	300		13161-28-9

250	45440	632-69-9	301	43800	603-45-2
251	50240	477-73-6	302	61554	17354-14-2
252	61552	6994-46-3	303	61565	128-80-3
253		7423-31-6	304	12055	842-07-9
305	12140	3118-97-6	328	26100	85-86-9
306	26105	85-83-6	329	26150	4197-25-5
307	11920	2051-85-6	330	26050	6368-72-5
308		123359-42-2	331		68504-35-8
309		23647-14-5	332		123333-78-8
310	45100	3520-42-1	333	45220	5873-16-5
311	19140	1934-21-0	334		4430-25-5
312		108321-10-4	335		1301-20-8
313		62637-91-6	336		123333-63-1
314		6262-21-1	337		386-17-4
315		632-73-5	338		4430-24-4
316		42798-98-1	339		1719-71-7
317	19540	1829-00-1	340	49005	2390-54-7
318	52000	78338-22-4	341		76-61-9
319		81012-93-3	342		125-20-2
320		123359-43-3	343	52040	92-31-9
321	12120	2425-85-6	344	14270	547-57-9
322	23850	72-57-1	345		14541-90-3
323	44045	2580-56-5	346	44040	2185-86-6
324	42595	2390-60-5	347	45190	6252-76-2
325		125-31-5	348		63721-83-5
326	16150	3761-53-3	349		14936-97-1
327		135-52-4	350		

One unique feature of these novel antiseptics is that they do not require another vehicle to attach to a surface. The adhesive potential of the dye makes them self-adhesive to surfaces of devices.

5 The antiseptic compound is therefore applied on the surface of a device by simply immersing the device in the antiseptic solution, air drying and washing out excessive antiseptic. The self-impregnating property of the dyes such as for example, the triarylmethane dyes, removes the need for another binding agent. This is another feature of the composition provided by this invention which is a considerable improvement over
10 other known compositions. Previously known compositions require other impregnating/coating agents and/or must typically be extruded into the device as it is made. Both these methods are time consuming and involve additional steps and techniques. For example, one method of coating devices first requires application or absorption of a layer of surfactant, such as tridodecylmethyl ammonium chloride (TDMAC) followed by the antibiotic coating layer, to the surface of the medical device.
15

Another method used to coat surfaces of medical devices with antibiotics involves first coating the selected surfaces with benzalkonium chloride followed by ionic bonding of the antibiotic composition (Solomon and Sherertz, 1987; U.S. Patent 4,442,133). Other methods of coating surfaces of medical devices with antibiotics are taught in U.S. Patent 5 4,895,566 (a medical device substrate carrying a negatively charged group having a pH of less than 6 and a cationic antibiotic bound to the negatively charged group); U.S. Patent 4,917,686 (antibiotics are dissolved in a swelling agent which is absorbed into the matrix of the surface material of the medical device); U.S. Patent 4,107,121 (constructing the medical device with ionogenic hydrogels, which thereafter absorb or ionically bind antibiotics); U.S. Patent 5,013,306 (laminating an antibiotic to a polymeric surface layer of a medical device); and U.S. Patent 4,952,419 (applying a film of silicone oil to the surface of an implant and then contacting the silicone film bearing surface with antibiotic powders). Furthermore, most of the methods previously employed to coat the surfaces of medical devices use antibiotics such as tetracyclines, penicillins, cephalosporins and the 10 beta-lactam antibiotics. The main drawback with antibiotics is the emergence of resistant strains.

Thus, the invention provides novel antiseptic derivative compounds with broad-spectrum antiseptic activity against bacteria and fungi including nosocomial and multidrug-resistant varieties with the additional ability to impregnate, bind, coat, adhere and/or attach 20 to various device surfaces without the assistance of impregnating vehicles such as tridodecylmethylammonium chloride (TDMAC). Furthermore, the novel antiseptic compounds of the invention also have an extended antimicrobial efficacy that can cover the life of the device.

One example of the novel broad-spectrum antiseptic derivatives of this invention is 25 gendine, which consists of the combination of gentian violet and chlorhexidine. Gentian violet, on its own, is a good impregnating triarylmethane dye. Bhatnager *et al.*, 1993 have shown in an *in vitro* study that gentian violet alone can be used to impregnate the surface of CSF silicone shunts and prevent the colonization of *S. epidermidis* on these surfaces. However, after impregnating the surfaces of various polymers, including polyvinylchloride, 30 gentian violet on its own has no activity against *Pseudomonas aeruginosa*, which is the second most common cause of nosocomial pneumonia and the third most common cause of

nosocomial urinary tract infections. Antiseptics such as chlorhexidine cannot attach on their own onto the surfaces of polyvinylchloride tubes or silicone catheters and silk sutures. They require an impregnating vehicle. Furthermore, on their own they are not highly active against *Pseudomonas aeruginosa*. On the other hand, upon the binding of gentian violet with chlorhexidine, the new antiseptic agent synthesized, is a potent and effective broad-spectrum antiseptic and has the additional ability to coat/impregnate various device surfaces. Gendine is unique in its ability to impregnate various device polymers, such as polyvinylchloride used in the formation of endotracheal tubes, silicone and polyurethane polymers used in the formation of vascular, as well as peritoneal, epidural, urinary and intraventricular catheters. In addition, gendine is able to impregnate the silk sutures used in surgical wounds.

In addition to **Gendine**, other antiseptics encompassed by this invention are **Genlenol** and **Genfoctol**.

The invention also provides methods to generate a wide variety of antiseptic medical devices. Some examples include antiseptic endotracheal tubes, antiseptic vascular catheters, including central venous catheters, arterial lines, pulmonary artery catheters, and peripheral venous catheters, antiseptic urinary catheters, antiseptic nephrostomy tubes, antiseptic biliary stents, antiseptic peritoneal catheters, antiseptic epidural catheters, antiseptic central nervous system catheters, including intraventricular shunts and devices, antiseptic prosthetic valves, orthopedic implants and antiseptic sutures.

B. Pathogens

The nosocomial bacterial infections result in diseases such as bacteremia, pneumonia, meningitis, osteomyelitis, endocarditis, sinusitis, arthritis, urinary tract infections, tetanus, gangrene, colitis, acute gastroenteritis, bronchitis, and a variety of abscesses, and opportunistic infections. Bacterial pathogens include Gram-positive cocci such as *Staphylococcus aureus*, coagulase negative staphylococci such as *Staphylococcus epidermidis*, *Streptococcus pyogenes* (group A), *Streptococcus spp.* (viridans group), *Streptococcus agalactiae* (group B), *S. bovis*, *Streptococcus* (anaerobic species), *Streptococcus pneumoniae*, and *Enterococcus spp.*; Gram-negative cocci such as *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Branhamella catarrhalis*; Gram-

positive bacilli such as *Bacillus anthracis*, *Corynebacterium diphtheriae* and *Corynebacterium* species which are diphteroids (aerobic and anaerobic), *Listeria monocytogenes*, *Clostridium tetani*, *Clostridium difficile*, *Escherichia coli*, *Enterobacter* species, *Proteus mirabilis* and other spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella*, *Shigella*, *Serratia*, and *Campylobacter jejuni*. The antibiotic resistant bacteria that can be killed by the antiseptic coated devices of the present invention include *Staphylococci* (methicillin-resistant strains), vancomycin-resistant enterococci (*Enterococcus faecium*), and resistant *Pseudomonas aeruginosa*.

Fungal infections that may be prevented include fungal infections (mycoses), which may be cutaneous, subcutaneous, or systemic. Superficial mycoses include tinea capitis, tinea corporis, tinea pedis, onychomycosis, perionychomycosis, pityriasis versicolor, oral thrush, and other candidoses such as vaginal, respiratory tract, biliary, eosophageal, and urinary tract candidoses. Systemic mycoses include systemic and mucocutaneous candidosis, cryptococcosis, aspergillosis, mucormycosis (phycomycosis), paracoccidioidomycosis, North American blastomycosis, histoplasmosis, coccidioidomycosis, and sporotrichosis. Fungal infections include opportunistic fungal infections, particularly in immunocompromised patients such as those with AIDS. Fungal infections contribute to meningitis and pulmonary or respiratory tract diseases.

Other pathogenic organisms that may be prevented from causing the infections include dermatophytes (*Microsporum canis* and other *M. spp.*; and *Trichophyton* spp. such as *T. rubrum*, and *T. mentagrophytes*), yeasts (e.g., *Candida albicans*, *C. Parapsilosis*, *C. glabrata*, *C. Tropicalis*, or other *Candida* species including drug resistant *Candida* species), *Torulopsis glabrata*, *Epidermophyton floccosum*, *Malassezia furfur* (*Pityrosporon orbiculare*, or *P. ovale*), *Cryptococcus neoformans*, *Aspergillus fumigatus*, and other *Aspergillus* spp., *Zygomycetes* (*Rhizopus*, *Mucor*), *hyalohyphomycosis* (*Fusarium* spp.), *Paracoccidioides brasiliensis*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidioides immitis*, and *Sporothrix schenckii*. Fungal infections include *Cladosporium cucumerinum*, *Epidermophyton floccosum*, and *Microspermum ypsorum*.

30

C. Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor 5 to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

10

EXAMPLE 1

Synthesis of Gendine and Impregnation of Devices

Impregnation Procedure

15 The general procedure involves, and when applicable, prior preparation of the basic reagent (such as chlorhexidine) in anhydrous solvent, addition of the basic reagent to a solution of a dye (such as Gentian violet) in anhydrous solvent (or addition of the dye to the basic solution), stirring the resulting mixture for 30-90 minutes at ambient conditions, evaporating the solvent also under ambient conditions, and finally dissolution 20 of the residue prior to impregnation. The following procedure illustrates impregnation with Gendine, an example for employing a basic guanidium derivative (e.g., chlorhexidine) and triarylmethane dye (e.g., Gentian violet).

Potassium *tert*-butoxide in THF, 7.35 ml of 1M solution, was added to a solution 25 of CHX diacetate, 1.533 g; 2.45mmol in 35 ml THF. The resulting heterogeneous solution was stirred for 20 minutes, then added to a solution GV, 1.0 g; 2.45 mmol, in 30 ml THF (GV used as an example of Triarylmethane Dye). The mixture was stirred at ambient conditions for 1 hour, then placed under the hood overnight to evaporate the solvent. The resulting residue was dissolved in 30 ml DCM (or MeOH). When applicable, the base (such as neutral form of chlorhexidine) is added to a stirring solution 30 of dye (such as GV) in DCM and the resulting mixture is stirred for at least 1 h. With anionic dyes, dissolution is achieved with the addition of at least one equivalent of a

quaternary amine (such as tetraethylammonium) prior to addition of the base. One-centimeter device segments were immersed in the DCM solution for the appropriate period, generally PVC and PU for 10 minutes; Silicone (Si) and Silk Suture for 2 hours. The devices were removed from the solution, and traces of solution were removed from
5 the lumen when applicable. The impregnated devices were placed under the hood to dry for at least 4 hours, preferably over night, then washed with distilled water until the washings were colorless or very faint, and finally placed under an aseptic hood to dry under ambient conditions for at least 4 hours, preferably overnight.

10 **In Vitro antimicrobial activity**

The antimicrobial activity of impregnated catheters was evaluated in duplicate by a modified Kirby-Bauer technique. BBL Mueller Hinton II agar plates (obtained from Fisher Scientific) were inoculated with 0.5 McFarland of the appropriate microorganism (hospital isolates from the MD Anderson Cancer Center). Then 10 mm segments of
15 impregnated devices were embedded in the inoculated plates and placed in an incubator at about 37°C for at least 18 hours. Zones of inhibition were measured perpendicular to the long axis of the device. Bioactivity against yeast constituted of two zones; a large outer zone of partial growth inhibition, and an inner smaller zone of complete inhibition. This later zone is reported herein.

20 **Results and discussion**

Tables 2 and 3 illustrate zones of inhibition obtained for Gendine-impregnated devices:

25

30

TABLE 2
Endotracheal PVC Tubes, (7.0 mm I.D.)

Zones of Inhibition (in mm) obtained for			
Reagent in DCM	<i>MRSA</i> ₂₀₆₆	<i>PS</i> ₄₂₀₅	<i>C. Parap.</i> 1-100-0022
GV [†]	25:25	0:0	27:27
CHX ^{††,§}	0:0	0:0	0:0
GN, 1 st trial [†]	28:28	21:21	27:28
GN, 2 nd trial [‡]	28:29	22:23	27:27

MRSA = Methicillin-Resistant *Staphylococcus aureus*.

PS = *Pseudomonas aeruginosa*

C. Parap. = *Candida Parapsilosis*

§DCM solution containing about 33% MeOH v/v.

[†]Device immersed for 10 minutes.

^{††}Device immersed for 2 hours.

[‡]Device immersed for 1 hour.

5

10

TABLE 3
Endotracheal PVC Tubes, (7.0 mm I.D.)

Zones of Inhibition (in mm) obtained for			
Reagent in MeOH	<i>MRSA</i> ₂₀₆₆	<i>PS</i> ₄₂₀₅	<i>C. Parap.</i> 1-100-0022
GV [†]	20:21	0:0	18:19
CHX [†]	0:0	0:0	0:0
GN [†]	24:25	13:13	23:23

[†]Device immersed for 2 hours.

15

As shown in Tables 2 and 3, endotracheal PVC tubes impregnated with Gendine (GN) are far more effective against all organisms when compared with those impregnated with CHX, and more effective than PVC tubes impregnated with GV against *Pseudomonas aeruginosa*.

20

TABLE 4
Double lumen 10.0 FR-Cook Silicone Catheter

Zones of Inhibition (in mm) obtained for			
Reagent in DCM	<i>MRSA</i> ₂₀₆₆	<i>PS</i> ₄₂₀₅	<i>C. Parap.</i> 1-100-0022
GV [†]	6:7	0:0	0:0
CHX ^{‡,§}	0:0	0:0	0:0
GN, 1 st trial [†]	18:19	11:12	19:19
GN, 2 nd trial [†]	19:19 (19:20) [‡]	10:11 (12:13) [‡]	18:18 (24:25) [‡]

MRSA = Methicillin-Resistant *Staphylococcus aureus*.

PS = *Pseudomonas aeruginosa*

C. Parap. = *Candida Parapsilosis*

[§]DCM solution containing about 33% MeOH v/v.

[†]Device immersed for 2 hours.

[‡]Values between parenthesis are for 20 hour immersions.

10 Again data in Table 4 shows how silicone catheters impregnated with GN are more effective in inhibiting *MRSA*, *PS* and *C. parapsilosis* than catheters impregnated with either GV or CHX.

TABLE 5
Double lumen 10.0 FR-Polyurethane Catheter

Zones of Inhibition (in mm) obtained for			
Reagent in DCM	<i>MRSA</i> ₂₀₆₆	<i>PS</i> ₄₂₀₅	<i>C. Parap.</i> 1-100-0022
GV [†]	22:22	0:0	22:23
CHX ^{‡,§}	17:17	10:10	15:15
GN, 1 st trial [†]	21:21	13:14	26:27
GN, 2 nd trial [‡]	22:22	15:15	22:23

MRSA = Methicillin-Resistant *Staphylococcus aureus*.

PS = *Pseudomonas aeruginosa*

C. Parap. = *Candida Parapsilosis*

[†]Device immersed for 10 minutes.

[‡]Device immersed for 2 hours.

[§]DCM solution containing about 33% MeOH v/v.

[‡]Device immersed for 1 hour.

25 Similarly, Table 5 indicates that PU catheters impregnated with GN are more effective than PU catheter impregnated with GV in inhibiting *Pseudomonas aeruginosa*, and more effective than PU catheters impregnated with CHX against all three organisms, *MRSA*, *PS* and *C. parapsilosis*.

TABLE 6
Silk Sutures

	Zones of Inhibition (in mm) obtained for		
Reagent	<i>MRSA</i> ₂₀₆₆	<i>PS</i> ₄₂₀₅	<i>C. Parap.</i> I-100-0022
GV [†]	8:8	0:0	0:0
CHX ^{‡,§}	0:0	0:0	0:0
GN, 1 st trial [†]	17:17	3:5	21:21
GN, 2 nd trial [†]	15:15	4:4	14:14

MRSA = Methicillin-Resistant *Staphylococcus aureus*.

PS = *Pseudomonas aeruginosa*

5 *C. Parap.* = *Candida Parapsilosis*

[†]Device immersed for 2 hours.

[‡]DCM solution containing about 33% MeOH v/v.

10 Table 6 as well shows that silk sutures coated or impregnated with GN are significantly more effective in inhibiting MRSA, PS and *C. parapsilosis* than sutures coated with either GV or CHX.

15 In addition to the simplicity of the impregnation procedure, and the availability of the requisite reagents, one unique feature of gendine-impregnated devices is its broad spectrum activity, not only against the worldwide problematic gram-positive MRSA, which has increased in frequency at an alarming rate as a cause of device-related infections, but also against *Pseudomonas aeruginosa*, an increasingly prevalent opportunistic human pathogen, and the most common gram-negative bacterium found in nosocomial infections. They are intrinsically more resistant than gram-positive bacteria to many antiseptics, particularly when present in a biofilm or when associated with a 20 device infection (Platt *et al.*, 1988).

25 For PVC and silicone (Si) devices, this invention shows no activity against *Pseudomonas aeruginosa*, when impregnated with either the immobilizing dye (gentian violet) or CHX alone. All devices impregnated with GN exhibit fair to good activity against *Pseudomonas aeruginosa*. This is important especially since *Pseudomonas aeruginosa* is responsible for 16% of nosocomial pneumonia cases (and is considered by the Centers for Disease Control as the second most common cause of nosocomial ventilator associated pneumonia), 12% of nosocomial urinary tract infections, 8% of surgical wound infections, and 10% of nosocomial bloodstream infections (Van Delden and Iglewski, 1998).

Staphylococcal resistance to antiseptics are known worldwide (Russel A. D., 1997). In addition to CHX, low-level resistance to three antiseptics (acriflavin, benzalkonium chloride, and hexamidine diisethionate is documented (Reverdy *et al.*, 1992; Townsend *et al.*, 1985; Heir *et al.*, 1995). The present study reveals that all GN-
5 impregnated devices, including sutures, exhibit significant biocidal activity against methicillin-resistant staphylococci. This finding is extremely important in light of the fact that methicillin-resistant staphylococci (MRSA and MRSE) are the leading causes of device-related infections, including vascular catheter-related bacteremia and surgical wound infections. In addition *S. aureus* is one of the leading causes of nosocomial
10 pneumonia (Klempner *et al.*, 1998).

The effectiveness of gendine-impregnated devices against *Candida* is no less noteworthy. As revealed from this study, silicone catheter and suture impregnated with GN exhibit fair to good activity against *C. Parapsilosis*, which is not the case for either GV or CHX-impregnated devices. Catheter-related candidemia is now the third leading
15 cause of vascular catheter-related bloodstream infections (Raad *et al.*, 1992). In addition, candidemia in severely immunocompromised patients (i.e., HIV, bone-marrow recipients and leukemia patients) is an important cause for morbidity and mortality and catheters are a major source for this infection (Tumbarello *et al.*, 1998; Gonzalez *et al.*, 1996; Lecciones *et al.*, 1992; Wey *et al.*, 1989). The known chlorhexidine-sulfadiazine
20 impregnated catheters and the minocycline-rifampin impregnated catheters do not have significant prophylactic effect against fungi (Tacconelli *et al.*, 1997; Raad *et al.*, 1997).

Other GV preparations

Antiseptics chloroxylenol [p-chloro-m-xylenol; 4-chloro-3,5-dimethylxylenol
25 (PCMx)], Clofoctol [α -2,4-dichlorophenyl)-4-(1,1,3,3-tetramethylbutyl)-o-cresol (CFTL), and Triclosan [2,4,4'-trichloro-2'hydroxydiphenyl ether] (TLS) are three of the phenolic antiseptic reagents included in this study. The first disinfectant is the first halophenol employed in many antiseptic and disinfectant formulations. Neither the neutral form of PCMx nor its sodium salt could produce *in vitro* zones of inhibition on
30 their own through coating or impregnating the catheter devices and sutures. However, when the salt is reacted with GV (as an example of a triarylmethane dye), the resulting

products (new products such as **Genlenol**, **Genlosan** and **Genfoctol**) are immobilized on the devices producing large in vitro zones of inhibition against various nosocomial pathogens. Meanwhile, Genlenol (GV⁺.PCM^X) not only increased the zone against MRSA considerably for the silicone catheter, but also produced a large zone against *C. parapsilosis*. Similar results were observed for silk sutures. Improvements in the zones of inhibition against *C. parapsilosis* are also observed for PVC tubes and PVC catheters, and to a large extent against *Alcaligenes faecalis* (a gram negative bacillary organism) for the PVC tubes. These results are summarized in Tables 7-10. Similar trends were observed for Genfoctol (GV⁺.CFTL⁻), but with much larger zones for sutures against MRSA and *C. parapsilosis*.

TABLE 7
Zones of Inhibition (in mm) imparted by endotracheal^f PVC tubes

Zones of Inhibition (in mm) against			
Reagent	<i>MRSA</i> ₂₀₆₆	<i>Alcaligenes faecalis</i> ₃₆₈₁	<i>C. Parap.</i> ₁₋₁₀₀₋₀₀₂₂
GV [†]	25:25	18:18	27:27
PCM ^X	0:0	0:0	0:0
GV ⁺ .PCM ^X [*]	27:27	27:28	34:34

^f7.0 mm I.D. [†]Gentian violet. [‡]Chloroxylenol. ^{*}Genlenol.

15
TABLE 8
Zones of Inhibition (in mm) imparted by Silicone catheters^f

Zones of Inhibition (in mm) against			
Reagent	<i>MRSA</i> ₂₀₆₆	<i>Alcaligenes faecalis</i> ₃₆₈₁	<i>C. Parap.</i> ₁₋₁₀₀₋₀₀₂₂
GV [†]	6:7	0:0	0:0
PCM ^X [‡]	0:0	0:0	0:0
GV ⁺ .PCM ^X [*]	16:16	0:0	16:16
CFTL [§]	0:0	0:0	0:0
GV ⁺ .CFTL	20:20	10:10	28:29

^fDouble lumen 10.0 FR-Cook Silicone catheter. [†]Gentian violet. [‡]Chloroxylenol. ^{*}Genlenol. [§]Clofoctol.
^{||}Genfoctol.

TABLE 9
Zones of Inhibition (in mm) imparted by Polyurethane catheters^f

Reagent	Zones of Inhibition (in mm) against		
	<i>MRSA</i> ₂₀₆₆	<i>Alcaligenes faecalis</i> ₃₆₈₁	<i>C. Parap.</i> ₁₋₁₀₀₋₀₀₂₂
GV [†]	22:22	18:18	22:23
PCM [‡]	0:0	0:0	0:0
GV [†] .PCM [‡] [*]	24:24	18:20	31:31
CFTL [§]	0:0	0:0	0:0
GV [†] .CFTL [§] ^{**}	23:23	15:17	30:32

^fDouble lumen 10.0 FR-catheter. [†]Gentian violet. [‡]Chloroxylenol. ^{*}Genlenol. [§]Clofoctol. ^{**}Genfoctol.

5

TABLE 10
Zones of Inhibition Imparted by Silk Sutures

Reagent	Zones of Inhibition (in mm) against	
	<i>MRSA</i> ₂₀₆₆	<i>C. Parap.</i> ₁₋₁₀₀₋₀₀₂₂
GV [†]	8:8	0:0
PCM [‡]	0:0	0:0
GV [†] .PCM [‡] [*]	11:11	5:5
CFTL [§]	0:0	0:0
GV [†] .CFTL [§] ^{**}	17:17	15:16

[†]Gentian violet. [‡]Chloroxylenol. ^{*}Genlenol. [§]Clofoctol. ^{**}Genfoctol.

10 In general, many other gentian violet basic preparations significantly affect the efficacy and biocidal activity of coated sutures and silicone-impregnated catheters against *MRSA* and *C. Parapsilosis*. Some examples are shown below in Table 11.

TABLE 11
Zones of Inhibition Imparted by Silicone Catheters and Silk Sutures

Reagent	Zones of Inhibition (in mm) against			
	<i>MRSA</i> ₂₀₆₆		<i>C. Parap.</i> 1-100-0022	
	Si-catheter	Silk Suture	Si-catheter	Silk Suture
GV [†]	6:7	8:8	0:0	0:0
GV [†] OH [‡]	18:19	17:17	19:19	13:14
GV [†] OCH ₃ [‡]	18:19	15:15	25:25	10:13
GV [†] Glycerin [‡]	19:20	13:14	18:18	9:9
GV [†] HEDTA ^{‡‡}	13:13	7:7	9:13	4:5
GV [†] TCSA [§]	14:14	12:13	11:11	10:10
GV [†] MBT [¶]	13:13	8:8	12:12	0:0

Gentian violet. [†]Trisodium n-(2-Hydroxyethyl)ethylenediaminetriacetate. [‡]TCSA= 3',4',5'-Trichlorosalicyl-anilide. [¶]MBT= 2-Mercaptobenzothiazole.

5

The above data clearly demonstrate significant improvement in the biocidal activity of impregnated or coated silicone catheters and silk sutures by the antiseptic derivatives of the invention.

10

EXAMPLE 2

Clinical trials

The antiseptic devices of the invention pose no significant risk. Hence, preclinical studies (animal studies) may not be required. This section is concerned with the development of human treatment protocols using the antiseptic medical devices of the present invention.

The various elements of conducting a clinical trial, including patient treatment and monitoring, will be known to those of skill in the art in light of the present disclosure. The following information is being presented as a general guideline for use in establishing the use of the antiseptic medical devices.

Candidates will be patients who are seriously ill and are required to use a medical device such as those described in the sections above. The medical devices in these cases will be treated with Gendine, Genelol, Genfoctol, Genlosan or other antiseptic derivatives

that can be synthesized by the methods provided herein, and the patients will be monitored for the occurrence of nosocomial infections.

To monitor the development of infections and to evaluate the efficacy of the antiseptic coated/impregnated medical devices in preventing the spread of infectious agents through the devices it is contemplated that the patients will be examined for appropriate tests every month. Tests that will be used to monitor the effectiveness of the treated medical device include: physical exam, X-ray, blood work and other clinical laboratory methodologies used to detect pathogens in the patients and also methods to detect presence of pathogens in the medical device. Described below is a study guideline for patients using central venous catheters.

A. Efficacy of Central Venous Catheters Coated with Antiseptics of the Invention

Patient Eligibility. Patients will be recruited from intensive care units, bone marrow transplant and melanoma services and other hospital divisions where catheters are used routinely on inpatients. Patients who require a new insertion of a central venous catheter (CVC) and have none of the exclusion criteria will be approached to obtain informed consent. The exclusion criteria are the following:

1. Age <18 years
2. Dermatitis over catheter insertion site
3. Pregnancy
4. Allergy to chlorhexidine or gentian violet
5. Expected duration of catheter placement <3 days
6. Inability to obtain informed consent

The eligible consenting patient will be informed that the catheter to be inserted has either been coated with an antiseptic compound (for example Gendine) or has not been coated, but the subject will not be informed as to whether the specific catheter to be inserted contains the compound.

Each female with child bearing potential will have a urine sample prior to catheter placement to test for pregnancy (if appropriate).

Catheter insertion. Catheters will be inserted into a subclavian vein or internal jugular vein using gown, mask, sterile gloves and full sterile drapes. Skin will be prepped using povidone iodine allowing 1 minute of exposure time. After insertion, the catheter will be secured to the skin using tape and the skin puncture site will be covered with povidone-iodine ointment. Then, the insertion site and the surrounding area will be covered with sterile gauze and taped securely.

Catheter maintenance. Catheters will be inspected every 72 hrs for evidence of site infection (erythema around catheter, purulent drainage, swelling tenderness over catheter). Every 72 hrs (or sooner if necessary) the dressing will be removed and the exit site will be re-prepped with povidone-iodine. All fluids, medications, etc. administered through each lumen will be documented.

Catheter types. The inventors contemplate using different types of catheters. For example, control catheters consist of triple lumen polyurethane catheters and single lumen polyurethane catheters will be tested among several others. The test catheters will be identical to the control catheters in appearance, but they will be coated with the antiseptics of the invention, for example, Gendine.

Trial design. The trial is a prospective randomized design. The patient, the health care worker inserting the catheter, the microbiologist culturing the catheter, and the evaluator will be blinded as to whether the catheter is coated or not coated with the antiseptics of the invention. They will, however, be identifiable by an assigned code number. After informed consent has been obtained a catheter will be pulled out of a box containing 6 test and control catheter placement trays. The boxes will consist of either triple or single lumen catheters and will be labeled as such. The trays will be placed in the boxes such that test and control catheters will alternate from top to bottom. Each box will contain 3 test and 3 control catheters. The unique identification number of the catheter will be recorded and will be included with the data analysis. Both the investigators and the patients will be blinded to the catheter identity throughout the study.

Statistical Considerations. Assuming a conservative baseline colonization and/or infection rate of at least 20% for central venous catheters, randomizing 75 patients to each arm would allow one to detect a change in catheter-related infection rates from 20% to 5% one sided significance and 80% power. If, after entering 150 patients, the

infection rate in the test arm has dropped by 50% (that is from 20% to 10%) then the study will be expanded to include 400 patients (200 in each arm). Using the selection criteria described above, the inventors estimate that they will test about 40 patients each month. Aiming for a total of 150 evaluable patients the study will be completed in approximately 6 months.

5 **Termination of Study.** Patients will be kept on study until the catheter is removed. The indication for catheter removal will be documented for each catheter. These include but are not limited to:

- 10 1. Catheter no longer needed
2. Catheter leaks
3. Bleeding around catheter
4. Catheter thrombosis
5. Catheter insertion site infection or sepsis
6. Positive blood cultures that are thought to be clinically significant (i.e. 15 associated fever, increased WBC) and no other site of infection is identifiable.

When the patient becomes febrile, blood will be withdrawn simultaneously through the lumen of the catheter and peripheral vein for quantitative blood culture. At the time of catheter removal, the catheter will be removed under aseptic conditions and 20 the tip and intracutaneous segments saved for culturing using the roll plate and sonication quantitative catheter culture technique. At the time of removal each lumen will be marked as to its prior use (hyperalimentation).

Patient Evaluation

25 1. **Pre-insertion evaluation.** Pertinent history will be taken and physical examination will be done regarding inclusion and exclusion criteria. Demographic data as well as details pertaining to underlying malignancy, treatment and antimicrobial treatment (including antimicrobial prophylaxis for infections in general in patients with hematologic malignancies) will be recorded. Investigational nature of study will be explained and informed consent will be obtained from patient. Pregnancy tests (serum or

urine) will be obtained on all female patients with child bearing potential. If the test is positive, the patient will be excluded.

Initial catheterization procedure details will be recorded including catheter type, site and date of placement; difficulty of insertion, and complications if any. The 5 difficulty of insertion will be determined by noting the following (a) number of attempts to insert the catheter (b) time spent during insertion (c) malpositioning and repositioning of a catheter.

2. **Post-insertion evaluation.** All patients will be monitored until the catheter is removed. Catheter site evaluation will be undertaken every 72 hrs with the 10 change of dressing. Special attention will be given to erythema, infiltration, pain, tenderness, swelling, suppuration, palpable cord in vessel, tissue warmth, lymphangitis or phlebitis. Details pertaining to chemotherapy, antineoplastic and antimicrobials, will be recorded. Catheter usage as for agents that might cause sclerosis of the vessel involved, hyperalimentation, blood and blood products administration, and drawing of blood will 15 be noted. The catheter insertion site will be recorded on every patient. In addition, events of repositioning the catheter after displacement will be recorded. Microbiologic evaluation of insertion site will be undertaken in the form of site cultures if suppuration is present. If catheter related septicemia is suspected, blood cultures will be drawn simultaneously through catheter and by peripheral venipuncture. Another set of cultures 20 will be drawn 24 hours later. If thrombophlebitis is suspected venous flow study of involved vessel will be done. If line related infection is suspected (including in patients with fever of unknown origin) or septicemia is documented, catheter will be changed over guide wire and distal as well as the proximal 5-7 cm of the catheter will be evaluated 25 for semiquantitative cultures by the inventors. The purpose of this procedure is diagnostic and not therapeutic. It will attempt to make a definitive diagnosis of catheter related infection by isolating the organism from the catheter using quantitative techniques.

3. **End of evaluation.** When it is decided to withdraw the line, the catheter 30 will be evaluated by the inventors for quantitative cultures. In addition, a quantitative blood culture will be drawn through the CVC lumen if the lumen is patent and peripherally if the patient is febrile.

Catheter Assessment

Definitions:

1. Catheter tunnel infection: Either the proximal and/or the distal catheter segments growing >15 colonies by the roll-plate culture technique or >100 colonies by the sonication culture technique.

5 2. Catheter exit site infection: development of lymphangitis, purulence or two of the following: erythema, tenderness, induration or warmth.

10 3. Catheter related septicemia: Recovery of same organism from catheter segment and blood without any other identifiable source for the septicemia. The catheter should grow at least 15 colonies of the organism by roll plate or at least 100 colonies by sonication. The patient should have clinical manifestation of sepsis (fever, chills or sudden hypotension).

15 4. Catheter-related infection: any of the conditions defined above would be considered as catheter-related infection.

15 Success will be measured if there is no catheter related infection and failure will be indicated by the presence of a catheter related infection.

20 **Adverse Reactions.** All patients will be monitored for an unexpected adverse reaction (e.g. increased inflammation, phlebitis) associated with the coated catheter, using a statistical sequential test method. The study will be stopped if major adverse reaction is identified. Otherwise, the study will continue until 75 patients in each group have been enrolled.

25 All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both 30 chemically and physiologically related may be substituted for the agents described herein

while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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